## REMARKS

### I. Status of the Claims

Claims 1-38 are pending in this application. Claims 30-38 have been withdrawn from consideration. Claims 1, 8, 11-14, and 16 have been amended as discussed in further detail below.

### II. Amendment to the Specification

The specification has been amended to include a description to pharmaceutically acceptable derivatives, including glycosides and active esters in the paragraph bridging pages 8-9. Support for this amendment can be found in claim 1 as originally filed at p. 26, lines 6-7. Accordingly, no new matter has been added by this amendment.

The specification has also been amended at p. 13, lines 1-3 by replacing the "□" symbol with a "μ" such that the specification refers to a dosage of "0.1μg to 100mg/kg of body weight per day, preferably in the range of 50μg to 20mg/kg/day." It is readily apparent that the appearance of a "□" is a typographical error where the "μ" symbol was not recognized by the font set. Such an error would not have happened with a member of the English alphabet. A "μ" would have been the most logical replacement since μg/kg/day is a common dosage in the pharmaceutical arts. Thus, it is believed that no new matter has been added by this amendment.

### III. Rejections under 35 U.S.C. § 112, first paragraph

Claims 1-29 stand rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the enablement requirement. (*Office Action* at pp. 2-3.) Applicants respectfully traverse this rejection.

**A.** The Examiner alleges that the "effectiveness of the claimed methods on the different types of diabetes cannot be ascertained" because the examples in the specification fail to state whether type 1 or type 2 diabetes is being treated. (*Id.* at p. 2.) The Examiner also doubts the effectiveness of the claimed methods to treat complications and associated conditions of diabetes, alleging a "highly unpredictable nature of treating various types of diabetes." (*Id.* at pp. 2-3.)

Applicants respectfully disagree. "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." (M.P.E.P. § 2164.01.).

Applicants respectfully submit that one of ordinary skill in the art would have known that diabetes and its complications and associated conditions arise from abnormal serum glucose levels. It is well known that maintaining normal serum glucose levels would alleviate these conditions. As evidence of the knowledge of one of ordinary skill in the art, Applicants submit a copy of Andreoli, T. E. et al. *Cecil Essentials of Medicine,* 4th Ed. Philadelphia, PA: W. B. Saunders Co., 1997, p. 536 ("Andreoli") to show that it would not require undue experimentation to determine the effectiveness of treatment of both type I and type II diabetes. Andreoli states that "treatment goals for patients with type I and type II diabetes are similar," although modified in certain circumstances. Treating both types of diabetes requires maintaining near-normal blood glucose levels, which "prevents the development and/or progression of diabetic complications."

The present specification suggests similar indicators, since Examples 5 and 6 demonstrate how treatment of diabetic rats by the claimed method "returned their serum glucose level to the normal." (*Specification* at pp. 5-6.) Thus, Examples 5 and 6 provide a test well known in the art to determine whether a composition would treat diabetes, regardless of whether it was type I or type II diabetes. Accordingly, the description and examples provided in the specification, combined with the knowledge of the skilled artisan, provide the requisite enablement of treating diabetes types I and II and their complications through attainment of near-normal blood glucose levels.

**B.** The Examiner also alleges lack of enablement in the use of the term "glycosidic [*sic*, glycosylic]." (*Office Action* at p. 3.) The Examiner contends that the term encompassed mono-, di-, and polysaccharides without providing guidance in how to select from among these choices. (*Id.*) Applicants respectfully traverse this rejection.

In determining enablement, it is noted that the specification need not teach what is already well known in the art. (M.P.E.P. § 2164.01.)

Applicants submit a copy of Loudon, M. *Organic Chemistry*, 3rd Ed. Redwood City, CA: Benjamin/Cummings Publishing Co., Inc., 1995, 1343-1348 ("Loudon"), an undergraduate chemistry textbook, to show that glycosylation of organic molecules is a synthetic technique well known in the art of organic chemistry. The submitted passages of Loudon provide several basic synthetic techniques for glycosylation.

Applicants also note that claim 1 of U.S. Patent No. 5,580,857 to Oden, which has been cited as a reference by the Examiner, refers to a glycoside substituent of a gibberrellin without the need for further clarification.

Given the knowledge in the field, Applicants respectfully submit that the skilled artisan would be able to synthesize the claimed glycosyl ethers and esters in a routine manner. Moreover, the specification at Examples 5-6 provides clear guidance on how to evaluate compounds of formula 1 for use in the claimed method , i.e., monitoring serum glucose levels. (*See, Specification* at pp. 22-23.) Such guidance, coupled with the knowledge in the art, have been shown to be sufficient evidence of enablement. *In re Wands*, 858 F.2d 731, 740 (Fed. Cir. 1988). Thus, the specification enables one of ordinary skill in the art to make and use the claimed invention through routine experimentation.

**C.** The Examiner also alleges lack of enablement in the use of the terms "allyl, aryl, arylalkyl, amidine, and unsaturated or saturated ring." (*Office Action* at p. 3.) Because these terms have not been limited to a range or carbon atoms, the Examiner contends that "it would take an undue amount of experimentation" to identify compounds with desired activity. (*Id.*)

In considering whether experimentation is undue, "time and difficulty of experiments are not determinative if they are merely routine." (M.P.E.P. § 2164.06.)

Here, Applicants respectfully submit that one of ordinary skill in the art would have appreciated that variation of the number of carbon atoms in allyl, aryl, arylalkyl, amidine, and unsaturated or saturated ring groups can be accomplished by numerous documented techniques. Many reagents used to form groups of these classes are commercially available in a variety of carbon atom lengths or ranges. As the specification provides clear procedures on how to evaluate such compounds for use in the claimed methods, one of ordinary skill in the art would not encounter an undue

amount of experimentation from the absence of a carbon number range on the cited substituent groups.

Accordingly, Applicants respectfully request withdrawal of these rejections.

## IV. Rejection under 35 U.S.C. § 112, second paragraph

Claims 1, 8, and 16 stand rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Applicants respectfully traverse this rejection.

The Examiner alleges that the use of the term "including" renders the claims indefinite. (*Office Action* at pp. 3-4.)

Applicants respectfully disagree. Under U.S. practice, the term "including" is synonymous with "comprising," which is construed as an open-ended transitional phrase. (M.P.E.P. § 2111.03.) Thus, use of the word "including" does not render claims 1 and 8 indefinite. However, to expedite prosecution, Applicants have amended line 2 of claims 1 and 8 to replace the term "including" with "comprising" without prejudice or disclaimer. Applicants have also amended the second to last line of claim 1 to delete "including lactones, glycosides, esters, active esters and salts thereof."

The Examiner also alleges that the use of the term "derivatives" renders claims 1, 8, 11, 17, and 29 indefinite. (*Office Action* at p. 4.) The Examiner also alleges that the specification "fails to provide definition of the term "derivatives." (*Id.*)

Applicants respectfully disagree. One of ordinary skill in the art would readily understand the meaning of the term "derivatives" based on the knowledge in the art and in view of the teachings of the specification. Moreover, the specification at the paragraph bridging pp. 8-9 (amended in this response to include "glycosides" and

"active esters") provides a definition of "derivatives." Accordingly, claims 1, 8, 11, 17, and 29 are sufficiently definite to comply with § 112, second paragraph.

Claims 13 and 14 are rejected because there is no antecedent basis in claim 1 for "related conditions." (*Office Action* at p. 4.) Applicants have amended claims 13 and 14 to replace "related conditions" with "associated conditions," as recited in claim 1.

Accordingly, Applicants respectfully request withdrawal of these rejections.

## V. Rejections under 35 U.S.C. §§ 102 and 103

Claims 17-29 stand rejected under 35 U.S.C. § 102(b) as being anticipated by, or in the alternative, under 35 U.S.C. § 103(a) as being obvious over U.S. Patent No. 5,580,857 ("Oden '857"), PCT Publication No. WO 96/20703 ("Wu"), WO 94/24260 ("Oden WO 94/24260") or EP 0 024 951 B1 ("Graebbe"). (*Office Action* at p. 5.) The Examiner alleges that each of these references "discloses the claimed gibberellins containing composition and a method for preparing the same." (*Id.*) The Examiner also contends that any differences between the claimed compositions and those of the cited references "would appear minor in nature," thereby rendering the claimed compositions *prima facie* obvious. (*Id.*) Applicants respectfully traverse these rejections.

***Wu***

Wu describes the use of gibberellin compounds and corresponding pharmaceutical compositions for treating wounds, ulcers, and lesions, and for cultivation of skin cell lines. (*Wu* at p. 3.) However, Wu does not disclose, teach, or suggest an anti-diabetic agent.

Applicants respectfully submit that "to anticipate a claim, the reference must teach every element of the claim." (M.P.E.P. § 2131.01.) Moreover, a *prima facie* case of obviousness is established if the prior art reference teaches or suggests every element of the claimed invention. (M.P.E.P. § 2143.01.) Applicants respectfully submit that these standards have not been met by the cited references.

Here, Claims 17-29, of which claims 17 and 19 are independent, are directed to an anti-diabetic agent. The term "anti-diabetic agent" does not simply recite an intended use but rather indicates the compounds that are suitable for administration to a diabetic patient. By the requiring an anti-diabetic agent, the present claims are a patentable selection among a broad range of known pharmaceutical compositions, many of which are not suitable as anti-diabetic agents. For example, a pharmaceutical composition comprising a pharmaceutically acceptable carrier such as glucose or sucrose would not be a suitable anti-diabetic agent and thus, would not be within the scope of claims 17-29.

Because Wu does not disclose anti-diabetic agents, Wu does not anticipate claims 17-29. Moreover, because Wu fails to teach or suggest compositions that would be suitable as anti-diabetic agents, Wu does not render obvious claims 17-29.

***Oden***

Oden '857 discloses the use of gibberellin compounds for the treatment of prostatitis and psoriasis. (*Oden '857* at col. 2, lines 31-34.) Oden WO 94/24260 discloses a composition "containing one or more gibberellins with activity against androgenic alopecia." (*Oden WO 94/24260* at p. 5.)

As discussed above, claims 17-29 are directed to anti-diabetic agents and thus, only encompass those compositions suitable as anti-diabetic agents. Oden '857 and Oden WO 94/24260 do not disclose, teach, or suggest the compound of formula (I) such that they would result in suitable anti-diabetic agents as claimed. Thus, claims 17-29 are patentable over Oden '857 and Oden WO 94/24260.

***Graebbe***

Graebbe is directed to "a new process for the production of gibberellins by fermentation." (*Graebbe* at col. 1, lines 1-4.) Graebbe, however does not disclose, teach, or suggest a pharmaceutically acceptable carrier or excipient, much less the combination of the anti-diabetic agent and pharmaceutically acceptable carrier or excipient as claimed. Thus, claims 17-29 are patentable over Graebbe.

***Davis***

Claims 1-29 stand rejected under 35 U.S.C. § 102(b) as being anticipated by, or in the alternative, under 35 U.S.C. § 103(a) as being obvious over Davis et al., *Journal of the American Podiatric Medical Association* (1989) 79:1, 24-26 ("Davis"). (*Office Action* at p. 6.) The Examiner cites Davis' composition comprising a gibberellin and its administration to diabetes-induced mice to allege that "treatment of diabetes would have been inherent from such an administration." (*Id.*) Applicants respectfully traverse this rejection.

Davis teaches a method of treating inflammation using a composition containing gibberellin. (*Davis* at p. 24.) The compositions were administered to diabetes-induced mice because of their poor healing and anti-inflammatory capabilities. (*Id.*) The mice

were then killed three hours after administration for analysis of inflammation activity. (*Id.* at p. 25.) Davis does not address the treatment of diabetes itself.

The Examiner alleges that Davis inherently treats diabetes. However, to establish the inherency, the allegedly inherently characteristic must necessarily flow from the teachings of the prior art reference. (M.P.E.P. § 2112.IV; *In re Robertson*, 169 F.3d 743 (Fed. Cir. 1999)). "The fact that a certain result or characteristic may occur or be present in the prior art is not sufficient to establish the inherency of that result or characteristic." *In re Rijckaert* 9 F.3d 1531, 1534, 28 USPQ2d 1955, 1957 (Fed. Cir. 1993) (emphasis in original).

Here, Davis does not explicitly or inherently disclose the claimed methods for treating diabetes and related conditions. Davis injected diabetes-induced mice with a gibberellin solution and sacrificed the mice for analysis 3 hours later. (*Davis* at p. 25.) Nowhere does Davis report on the degree of diabetes in the mice before or after administration of the composition, nor does Davis teach that conditions such as the disclosed dosage and time period between administration and killing the mice are sufficient to treat diabetes. The allegation that Davis' method inherently discloses the claimed method of treating diabetes is without a reasonable basis because the Examiner has provided no facts or technical reasoning to explain how Davis' disclosure would necessarily achieve the presently claimed method of treating diabetes.

Moreover, the claimed method requires that the composition be administered to a patient "in need thereof," i.e., in need of treatment of diabetes. The significance of this claim limitation in giving weight to a preamble has been deliberated by the Federal Circuit. *Christian J. Jansen, Jr. v. Rexall Sundown, Inc.*, 342 F.3d 1329 (Fed. Cir.

2003). In *Jansen*, the patent at issue had two independent claims directed to "a method of treating or preventing macrocytic-megaloblastic anemia ... which comprises administering a daily oral dosage of a vitamin preparation to a human in need thereof." *Id.* at 1330. In construing the claims, the Federal Circuit gave weight to the ordinary meaning of the preamble "for treating or preventing macrocytic-megablastic anemia" because it set forth an objective connected to the limitation "to a human in need thereof," which was presented in the body of the claim. *Id.* at 1333. The Federal Circuit discussed the effect of giving weight to the combination of these limitations:

> Finally, that "need" must be recognized and appreciated, for otherwise the added phrases do not carry the meaning that the circumstances of their addition suggest that they carry. In other words, administering the claimed vitamins in the claimed doses for some purpose other than treating or preventing macrocytic-megaloblastic anemia **is not practicing the claimed method**, because Jansen limited his claims to treatment or prevention of that particular condition in those who need such treatment or prevention.

*Id.* at 1334 (emphasis added).

Similar to the *Jansen* claims, independent claims 1 and 8 recite a "method for treatment of diabetes and its complications and related conditions" in the preamble, followed by the term "in need thereof," in the body of the claim. Like *Jansen*, claims 1 and 8 recite an "intentional purpose" for treating the claimed condition, and thus, methods of treating conditions other than "diabetes and its complications and associated conditions" are not encompassed by claims 1-8.

Claims 11 and 12 have been amended to add the limitation "to a patient in need thereof." Thus, amended claims 11 and 12 must be construed in a manner consistent

with the holding in *Jansen*, i.e., that the claims encompass a method of treating diabetes and its complications and associated conditions.

Moreover, Davis does not teach or suggest a method of treating diabetes and its complications and associated conditions, as claimed. Rather, Davis teaches compositions for use in anti-inflammatory purposes. Thus, Davis does not present a *prima facie* case of obviousness over the claimed method.

Finally, Davis does not disclose, teach, or suggest an anti-diabetic agent as recited in claims 17-29 for the same reasons discussed above for Wu.

***Wu***

The Examiner has rejected claims 1-29 under 35 U.S.C. § 102(b) as being anticipated by, or in the alternative, under 35 U.S.C. § 103(a) as being obvious over PCT Publication No. WO 96/20703 ("Wu"). (*Office Action* at p. 6.) The Examiner believes Wu's compositions and methods comprising gibberellins for treating ulcers and wounds are encompassed by the claimed methods of treating complications and associated conditions of diabetes. (*Id.*) Applicants respectfully traverse this rejection.

The rejection of claims 17-29 over Wu has been addressed above.

Wu does not describe all of the present claim limitations of claims 1-16, namely that of a method of treating diabetes and its complications and associated conditions comprising administering compounds of formula (1) to a patient in need thereof. As discussed above, Applicants respectfully submit that "a method of treatment for diabetes and its complications and associated conditions" together with the limitation "in need thereof" is anticipated only if the reference discloses such treatment. Because Wu

does not disclose, teach, or suggest the claimed methods. Wu does not anticipate or render obvious the claimed method.

Accordingly, Applicants respectfully request withdrawal of these rejections.

## VI. Conclusion

In view of the foregoing amendments and remarks, Applicant respectfully requests reconsideration and reexamination of this application and the timely allowance of the pending claims.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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Dated: April 26, 2005

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# Cecil Essentials of Medicine

Fourth Edition

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tes. As B cell mass declines, insulin secretion decreases until the available insulin is no longer adequate to maintain normal blood glucose levels. Although the specific genes related to type I diabetes have not been found, patients with type I diabetes are more likely to express DR3 and/or DR4 class II HLA molecules. (About 90 to 95% of patients with type I diabetes compared with 50 to 60% in the general population have these HLA haplotypes.)

Treatment with immunosuppressive agents in patients with new-onset type I diabetes has been attempted to prevent the ongoing immune-mediated destruction of B cells. This has been found to be effective, although diabetes returns immediately upon cessation of the immunosuppressive agent; the side effects and risks of long-term immunosuppression are felt to be greater than the risk of diabetes. Therefore, this therapy is not routinely employed. Another approach is to start children considered high risk for developing type I diabetes (i.e., those with positive antibodies, appropriate haplotype, decreased acute-phase insulin release but normal FPG) on low doses of insulin. This technique can delay the complete obliteration of the pancreas's ability to secrete insulin and allows the patient to have more years with less "brittle" diabetes (the presence of endogenous insulin makes treatment of diabetes much easier).

After the initial diagnosis, patients with type I diabetes often undergo a "honeymoon" period, in which the ability to secrete endogenous insulin returns transiently before it is lost forever. It is important to monitor patients clinically for the development of the honeymoon period, because insulin doses usually need to be decreased (and sometimes stopped) based on the patient's SMBG results. This period can last for up to 1 year, but once it ends, it is usually necessary to begin increasing the patient's insulin doses to maintain near-euglycemia.

## Type II Diabetes

Type II diabetes has a powerful genetic predisposition (90 to 100% concordance in identical twin pairs), although the exact genetic basis is unknown. It is likely that more than one pathogenic mechanism will be found. Many patients with type II diabetes are asymptomatic, and their diabetes is either diagnosed during screening for diabetes or when the patient is seen for another, unrelated medical problem. Patients who develop type II diabetes exhibit peripheral insulin resistance along with insufficient pancreatic B cell secretion of insulin. As hyperglycemia develops, glucotoxicity occurs, which further decreases insulin secretion. The liver also is resistant to the inhibitory effects of insulin, and as a result, hepatic gluconeogenesis is not adequately suppressed, leading to fasting hyperglycemia. Most patients (90%) who develop type II diabetes are obese, and obesity itself is associated with insulin resistance, which further worsens the diabetic state.

Type II diabetes is becoming increasingly common because more people are living longer (diabetes increases with age). It is also occurring more frequently in younger people, as more individuals are exposed to high-calorie Western diets, leading to childhood obesity.

# TREATMENT OF DIABETES

## Treatment Goals

The treatment goals for patients with type I and type II diabetes are similar, although they are modified in certain circumstances (e.g., advanced age, hypoglycemia unawareness). Table 71-4 outlines recommended treatment guidelines. The Diabetes Control and Complications Trial (DCCT) demonstrated that maintaining blood glucose concentrations in a near-normal range prevents the development and/or progression of diabetic complications. Intensive treatment, with maintenance of an $HgbA_{1c}$ level of ~7.0% and an average blood glucose concentration of ~150 mg/dl, decreases the development of clinically significant diabetic retinopathy by 76%, proteinuria by 54%, and clinical neuropathy by 60%, as compared with incidence in the patients with less intensive ("standard") insulin therapy. However, intensive treatment is associated with a marked increase in episodes of hypoglycemia; this is one of the largest obstacles faced by patients striving to maintain near-normal blood glucose levels.

The findings of the DCCT are also thought to be applicable to patients with type II diabetes because there is no difference in the pathophysiology of development of the microvascular and neuropathic complications in either type of diabetes. However, controversy exists over the putative greater danger of hyperinsulinemia in type II diabetes as well as the greater risk of hypoglycemia in patients likely to have macrovascular disease. For young patients with type II diabetes, however, the risks of developing the microvascular and neuropathic complications of diabetes probably outweigh the risks associated with hyperinsulinemia and hypoglycemia.

When treating diabetes, glycated hemoglobin levels (such as $HgbA_{1c}$) are the best indicator of overall diabetic control, reflecting the average blood glucose levels over the past 8 to 12 weeks. Different assays are used to measure glycated hemoglobin levels, with differing normal ranges, so it is important to become familiar with the assay used locally. For clinical purposes, an acceptable $HgbA_{1c}$ level is <1.5% above the upper limit of normal of the assay used. Therefore, if the normal range (in nondiabetic controls) for the $HgbA_{1c}$ is 4.0 to 6.2%, an acceptable target for blood glucose control is $\leq 6.2\% + 1.5\% = 7.7\%$. An extremely motivated patient may wish to work to maintain an $HgbA_{1c}$ level of 7.0% (similar to the control in the DCCT trial); with proper management, this goal can often be achieved. Preprandial blood glucose levels should be 80 to 140 mg/dl (and can be 70 to 120 mg/dl in a patient trying hard to achieve near-euglycemia). The prebedtime snack blood glucose target level in patients taking insulin should be 100 to 140 mg/dl to avoid nocturnal hypoglycemia.

Some patients should not aim for near-normal blood glucose levels. In elderly patients who have a life expectancy of <5 years or in any patient with a terminal dis-

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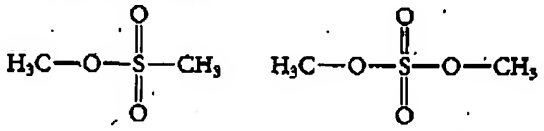
the synthesis of DNA and RNA fragments also exist. These methods strategically resemble peptide synthesis in the sense that a strand of DNA or RNA is "grown" on a solid support from individual nucleotides by using a series of protection, coupling, and deprotection steps. Molecular biologists have also discovered ways in which foreign DNA can be incorporated into, and expressed by, host organisms. All of these techniques used together have led to new biotechnologies that have been termed collectively "genetic engineering." One major pharmaceutical house employs a lowly bacterium—*Escherichia coli*—for the commercial production of *human* insulin using these techniques. Formerly all insulin used for the treatment of diabetes came from horses and pigs, and shortages of this important hormone occurred. The new process has made available an abundant supply of human insulin and was one of the first of many such processes that have been developed for the production of complex biological materials for use in human medicine.

As this edition of this text was being prepared, the sequencing of the human genome was completed. That is, the DNA sequence of all human DNA is now known. Now scientists must learn to interpret—or, more accurately, how the living human system interprets—this vast amount of information. For what proteins does the DNA code? What is the role of these proteins, some of which are unknown? How is DNA transcription affected by external stimuli? These questions are the domain of new sciences called *genomics* and *proteomics*. It is not hard to envision the day in which humankind will have at hand all the knowledge necessary to exert the type of control over gene expression that is needed to cure some diseases for which no cures presently exist.

## B. DNA Modification and Chemical Carcinogenesis

We've shown how the double-helical structure of DNA, DNA replication, and the fidelity of DNA transcription into RNA involve very specific base-pairing complementarity. Other important processes, such as the recognition of the three-base triplet code of mRNA during protein biosynthesis, also involve this type of complementarity. The molecular basis of this complementarity is the specific hydrogen bonding between a pyrimidine and a purine base. You can perhaps imagine that, if this hydrogen bonding were upset, the base-pairing complementarity would also be upset, and with it, some or all of the biological processes that rely on this phenomenon. There is strong circumstantial evidence that chemical damage to DNA can interfere with this hydrogen-bonding complementarity and can in some cases trigger the state of uncontrolled cell division known as cancer.

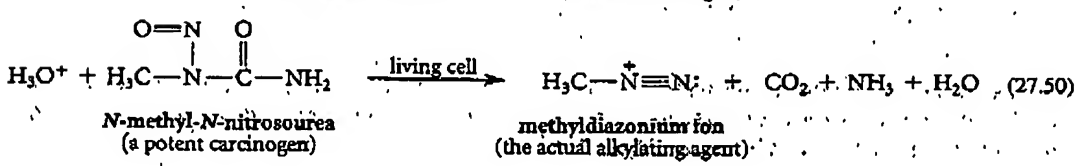
One type of chemical damage to DNA is caused by *alkylating agents* (Sec. 10.3B). Certain types of alkylating agents react with DNA by alkylating one or more of the nucleotide bases. These same alkylating agents are also *carcinogens* (cancer-causing compounds). A few such compounds are the following:



methyl methanesulfonate (a weak carcinogen)



dimethyl sulfate (a weak carcinogen)

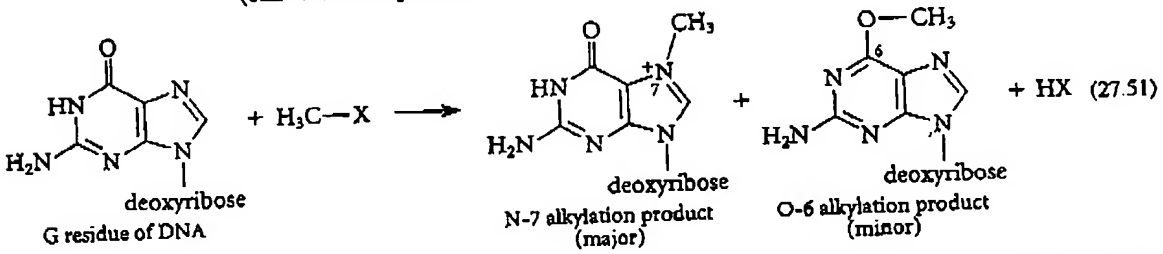


*N*-methyl-*N*-nitrosourea (a potent carcinogen)

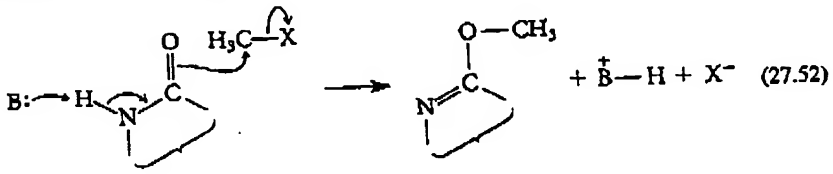
methyldiazonium ion (the actual alkylating agent)

(27.50)

When such alkylating agents (abbreviated $H_3C—X$ in the following equations) react with DNA, alkylated guanosines are among the products. The major product is alkylated on N-7 of the guanine base, but an important minor product is alkylated on the oxygen at C-6 (called the O-6 position).

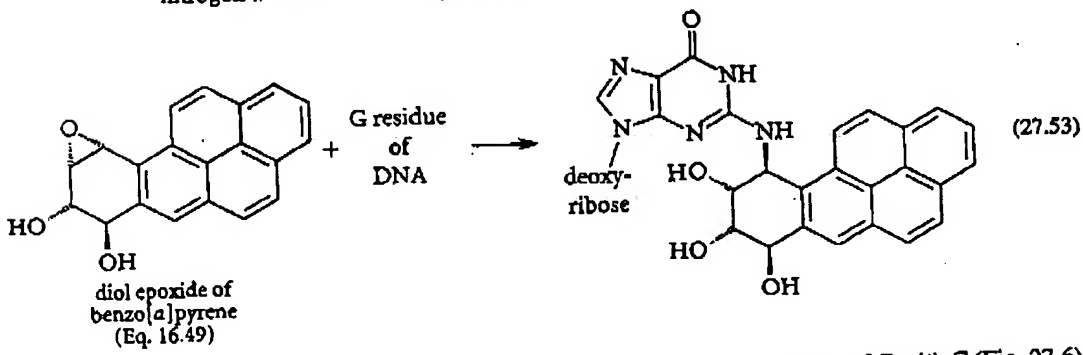



(An analogous alkylation occurs at O-4 of thymine; see Problem 27.33.) Notice that the alkylation at O-6 prevents the N-1 nitrogen from acting as a hydrogen-bond donor in a Watson-Crick base pair (Fig. 27.6) because the hydrogen is lost from this nitrogen as a result of alkylation. (B: = a base.)



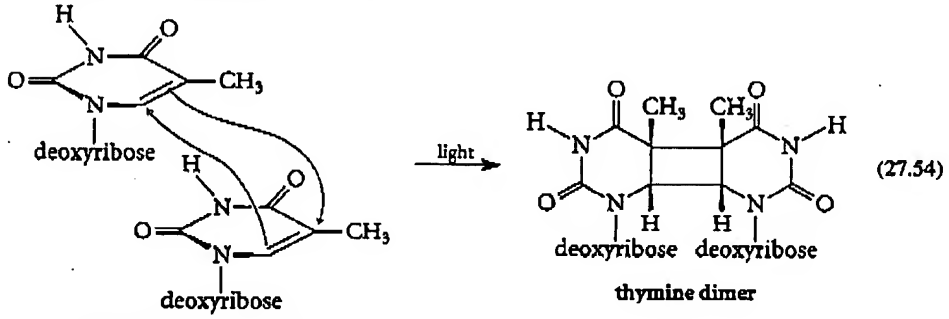

The N-7 alkylation, in contrast, does not directly affect any of the atoms involved in the hydrogen-bonding complementarity. It has been found that the alkylating agents which are the most potent carcinogens also yield the greatest amount of the guanines alkylated at O-6 and thymines alkylated at O-4. Although this correlation does not prove that these alkylations are primary events in carcinogenesis, it provides strong circumstantial evidence in this direction.

The way in which aromatic hydrocarbons are converted into carcinogenic epoxides by enzymes in living systems was discussed in Sec. 16.7. These epoxides have been shown to react with DNA; among the products of this reaction is a guanosine residue alkylated on the nitrogen at carbon-2 of the guanine base.




This nitrogen is also involved in the hydrogen-bonding interaction of G with C (Fig. 27.6). Thus, it may be that alkylation by aromatic hydrocarbon epoxides also triggers the onset of cancer by interfering with the base-pairing complementarity.

One last example of DNA damage is caused by ultraviolet radiation. Ultraviolet light promotes the [2*s* + 2*s*] cycloaddition (Sec. 25.3) of two pyrimidines when they occur in adjacent positions on a strand of DNA. In the following example, a thymine dimer is formed from two adjacent thymines.




two thymines in DNA at adjacent positions on same strand

thymine dimer

(27.54)

Most people have a biological repair system that effects the removal of the modified pyrimidines and repairs the DNA. People with a rare skin disease, *xeroderma pigmentosum*, have a genetic deficiency in the enzyme that initiates this repair. Most of these people contract skin cancer and die at an early age. Here, then, is a situation in which the chemical modification of DNA has been clearly associated with the onset of cancer.

As these examples show, it is possible to understand the molecular basis of some diseases. Certainly further progress in human medicine will stem from an understanding of the organic chemistry of the living cell.

## PROBLEM

27.33 There is evidence that alkylation at O-4 of thymine, like alkylation at O-6 of guanine, is another mutagenic event that can lead to cancer.
(a) Draw the structure of a thymine residue as it would exist after O-4 methylation.
(b) Explain why O-4 alkylation at thymine would disrupt Watson-Crick base pairing.

## KEY IDEAS IN CHAPTER 27

- Carbohydrates are aldehydes and ketones that contain a number of hydroxy groups on an unbranched carbon chain, as well as their chemical derivatives.
- The D,L system is an older but widely used method for specifying carbohydrate enantiomers. The D enantiomer is the one in which the asymmetric carbon of highest number has the same configuration as (*R*)-glyceraldehyde (D-glyceraldehyde).
- Monosaccharides exist in cyclic furanose or pyranose forms in which a hydroxy group and the carbonyl group of the aldehyde or ketone have reacted to form a cyclic hemiacetal.
- The cyclic forms of monosaccharides are in equilibrium with small amounts of their respective aldehydes or ketones and can therefore undergo a number of aldehyde and ketone reactions. These include oxidation (bromine water or dilute nitric acid); reduction with sodium borohydride; cyanohydrin formation (the first step in the Kiliani-Fischer synthesis); and base-catalyzed enolization and enolate-ion formation (the Lobry de Bruyn-Alberda van Eckenstein reaction).
- The —OH groups of carbohydrates undergo many typical reactions of alcohols and glycols, such as ether formation, ester formation, and glycol cleavage with periodate.

- Because the hemiacetal carbons of monosaccharides are asymmetric, the cyclic forms of monosaccharides exist as diastereomers called anomers. The equilibration of anomers is why carbohydrates undergo mutarotation.
- In a glycoside the —OH group at the anomeric carbon of a carbohydrate is substituted with an ether (—OR) group. In disaccharides or polysaccharides, the —OR group is derived from another saccharide residue. The —OR group of glycosides can be replaced with an —OH group by hydrolysis. Thus, higher saccharides can be hydrolyzed to their component monosaccharides in aqueous acid.
- Disaccharides, trisaccharides, and so on, can be classified as reducing or nonreducing sugars. Reducing sugars have at least one free hemiacetal group. In nonreducing sugars all anomeric carbons are involved in glycosidic linkages.
- Ribonucleotides and deoxyribonucleotides, which are phosphorylated derivatives of ribonucleosides and deoxyribonucleosides, are the building blocks of RNA and DNA, respectively. These compounds are $\beta$ derivatives of either ribose or 2′-deoxyribose, respectively, and a purine or pyrimidine base. Adenine, guanine, and cytosine are bases in both DNA and RNA; thymine is unique to DNA, and uracil to RNA.
- An important conformation of DNA is the double helix, in which two right-handed helical strands of DNA running in opposite directions wrap around a common axis. The sugars and phosphate groups lie on the outside of the helix, and the bases are stacked in parallel planes on the inside. The two strands of the double helix are held together by purine-pyrimidine hydrogen bonds between complementary residues. A number of known carcinogens apparently modify DNA in such a way that this complementary hydrogen bonding is disrupted.

Reaction Review

*For a summary of reactions discussed in this chapter, see Section R, Chapter 27, in the* Study Guide and Solutions Manual.

## ADDITIONAL PROBLEMS

**27.34** Give the product(s) expected when D-mannose (or other compound indicated) reacts with each of the following reagents. (Assume that cyclic mannose derivatives are pyranoses.)
(a) $Ag^+(NH_3)_2$
(b) dilute HCl
(c) dilute NaOH
(d) $Br_2/H_2O$, then $H_3O^+$
(e) $CH_3OH$, HCl
(f) acetic anhydride
(g) product of part (d) + $Ca(OH)_2$, then $Fe(OAc)_3$, $H_2O_2$
(h) product of part (e) + $PhCH_2Cl$ (excess) and NaOH

**27.35** Give the products expected when D-ribose (or other compound indicated) reacts with each of the following reagents.
(a) dilute $HNO_3$
(b) $^-CN$, $H_2O$
(c) product of part (b) + $H_2/Pd/BaSO_4$ + $H_3O^+/H_2O$
(d) $CH_3OH$, HCl (four isomeric compounds; two pyranosides and two furanosides)
(e) products of part (d) + $(CH_3)_2SO_4$ (excess) and NaOH

**27.36** Draw the indicated type of structure for each of the following compounds.
(a) CDP (cytosine diphosphate; sugar ring in Haworth projection)
(b) α-D-talopyranose (chair)
(c) propyl β-L-arabinopyranoside (chair)
(d) (+)-lactose (Haworth projection)

**27.37** Name the specific form of each aldose shown here.
(a)